for the untreated control. The stems and roots were removed one week after treatment. The stem and roots were collected and stored at 4° C until the extraction process was performed.

Sample Preparation

The samples from the summer field comparison site were cut into 4 sections using hand-held clippers: main stem (MS: from soil line to cut surface); lower stem (SS: from soil line to branching of main roots); major roots (MR: major/large diameter roots); and fine roots (FR). The fine roots were removed and collected from the red maple samples and the fall field comparison site and ground using a Foss Cyclotec Model 1093 grinder with a 0.5 mm mesh screen. For the other plant tissue, a hand held saw was used to make cuts on three different locations of the samples: one inch below the cut (MS), at the soil surface (SS), and on the main root (MR). The saw dust from each sample was collected.

Extraction and Analysis

Extraction process was modified from the method by Wendelburg and Olberding (2008) and Olberding, et al., (1997). Tissue was extracted (1 g tissue : 20 ml extraction solvent) in a methanol/2.5 N NaOH (90:10) solvent, in a blender for 60 seconds, and then centrifuged at 2000 rpm for 5 min. One ml of the supernatant was combined with 2 ml of 0.5 N HCl, which was then stored at 4° C. The extracted tissue was subjected to solid phase and liquid phase partitioning prior to analysis via GC-MS. The solid phase partitioning utilized a Phenomenex Strata-X 30-mg SPE column. The column was equilibrated with 1 mL of acetonitrile, followed by 1 mL of 0.5 N hydrochloric acid, and dried under full vacuum for 5 sec between solvents. The sample was transferred in two 750 µl aliquots to the column at a flow rate of 1 ml/min, and the eluate discarded. The column was then rinsed with two 750-µL aliquots of an acetonitrile/water/1 N hydrochloric acid (30:69:1) solution, and the eluate discarded. The column was then dried under full vacuum for 10 min. The triclopyr was eluted from the solid phase partitioning column with two 500-µL aliquots of an acetonitrile/water/1 N hydrochloric acid (60:39:1) solution, under full vacuum for 30 seconds after each aliquot, and collected in a screw-top vial. The samples were capped and stored in at -20 C.

For liquid phase partitioning, 4 ml H_2O was added to the eluted sample. The sample was partitioned three times with 3.0 ml of dichloro-methane (DCM) (9.0 ml total). For each phase separation, the DCM was added, the vial was capped, vortexed for 20 sec, and centrifuged for two min for complete phase separation. The combined DCM fractions were dried with sodium sulfate (Na2SO4) for 24 hrs. To derivatize the samples, they were initially evaporated to dryness under a stream of N2 at 40° C, and 200 µL of derivatization solvent [acetonitrile with TFA (Trifluoroacetic acid), NMM (N-methylmorpholine), and ISTDs (dibutylpyridine, 4-butylphenol, and octyl-B-D-glucoside)] was added. This was followed by the addition of 50 µL of derivatization reagent [10% HMDS (Hexamethyldisilazane) in TMSDMA (N-Trimethylsilyldimethyl-amine)]. The samples were then heated to 60° C for 1 hour. The derivatized samples were then transferred to GC vials.

The samples were analyzed utilizing an Agilent GC-MS (HP 5890-HP 5971). The run conditions included an inlet temp of 275˚C, a flow rate 7.7 psi (36.3 cm/sec), and an injection volume of 1 µl (splitless). The run program began at 50˚C for 0.5 min, then 50˚C to 80˚C at 15˚C/min, then 80˚C to 320˚C at 10˚C/min; ending the run at 320˚C for 5 min. The total runtime was 31.5 min. The detector operated at 300˚, and the column was a Restek Rtx-5MS (Crossbond 5% diphentl - 95% dimethyl polysiloxane) 30 m x 0.25 mm ID x 0.25 µm df.